

30. A process according to any of claims 1-29 wherein the protein is capable of having at least 1, such as at least 2, at least 3, at least 4, at least 5, or at least 6 disulphide bonds.

31. A process according to any of claims 1-30 wherein the protein is capable of having at most 20, such as at most 15, at most 10, at most 8, at most 5, at most 4, at most 3, or at most 2 disulphide bonds.

32. A process according to any of claims 3-31 wherein the cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell and a plant cell.

10

33. A process according to any of claims 1-32 wherein the cell is a bacterial cell selected from the group consisting of a gram positive bacterium and a gram negative bacterium.

34. A process according to claim 33 wherein the gram negative bacterium is *E. coli* including a strain BL21 or a derivative thereof or a strain XA90 or a derivative thereof.

15

35. A process according to any of claims 1-34 wherein the cell is genetically modified to have a less reducing intracellular environment than a non-modified cell of the same strain.

36. A process according to any of claims 1-35 wherein the cell has been modified to have a reduced or lacking activity of a thioredoxin reductase or an enzyme having a similar effect on the sulfhydryl reducing potential of the cytoplasm.

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37. A process according to any of claims 1-36 wherein the modified cell is a *trxB*- mutant.

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38. A process according to any of claims 1-37 wherein the gene is a derivative of a naturally occurring gene.

39. A process according to claim 38 wherein the derivative is obtained by substituting at least one codon which is used more frequently by the host cell than the one originally present where the codon codes for the same amino acid.

30

40. A process according to any of claims 1-39 wherein the gene is under control of a regulatory DNA sequence not naturally associated with the gene.

35

41. A process according to any of claims 1-40 wherein the bacterial cell is transformed with an expression vector selected from the group consisting of pET vectors, e.g. T7 promoters.
- 5 42. A stable peptide free MHC protein obtainable by a process according to any of claims 1-41.
43. A kit comprising a MHC class I heavy chain and a  $\beta_2m$  allowing the recipient to produce and measure or detect a functional MHC class I protein to which a peptide, which is
- 10 capable of binding to said MHC class I protein, can be added leading to the generation of a functional MHC class I protein.
44. A kit according to claim 43 which comprises labelling of one or more MHC class I sub-units (heavy chain,  $\beta_2m$  and/or peptide) to measure or detect the generation of the MHC
- 15 class I protein.
45. A kit according to claim 43 and 44 wherein the measurement or detection system of the generated MHC class I protein is selected from the group of technologies consisting of radio-ligand, immuno-precipitation, ELISA, plasmon resonance, fluorescence polarization,
- 20 analytical ultracentrifugation, biochemical precipitation, ultrafiltration, chromatography and equilibrium dialysis.
46. A kit according to claims 43-45 which comprises an oligomerization of MHC proteins, such as two, three, four or more.
- 25 47. A kit according to claims 43-46 wherein a further reagent is added as a marker making the kit suitable for diagnostic purposes.
48. A kit according to claim 44-47 wherein the marker is selected from the group consisting of biotin, fluorochromes, enzymes, chemiluminescence, and radioactive markers.
- 30 49. Use of a process according to any of claims 1-41 in the manufacturing of MHC.

50. Use of a stable empty MHC protein according to claim 42 in analysis of the effect of changing an amino acid in the MHC on the binding specificity of said MHC as assessed by an analysis using a peptide library approach be it a synthetic or recombinant library.

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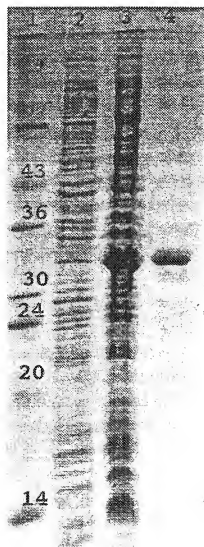


Fig. 1

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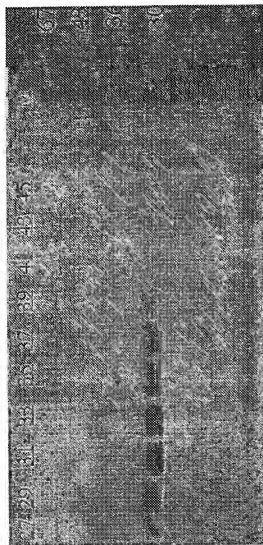


Fig. 2

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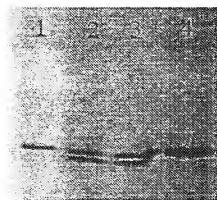


Fig. 3

Fig. 3

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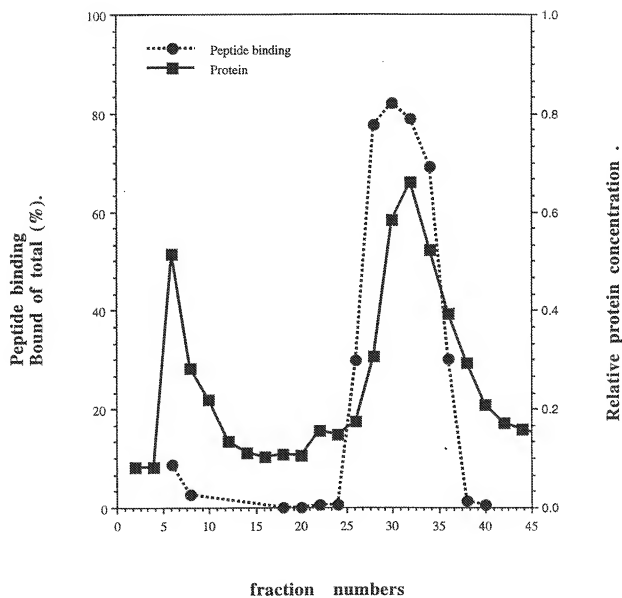


Fig. 4

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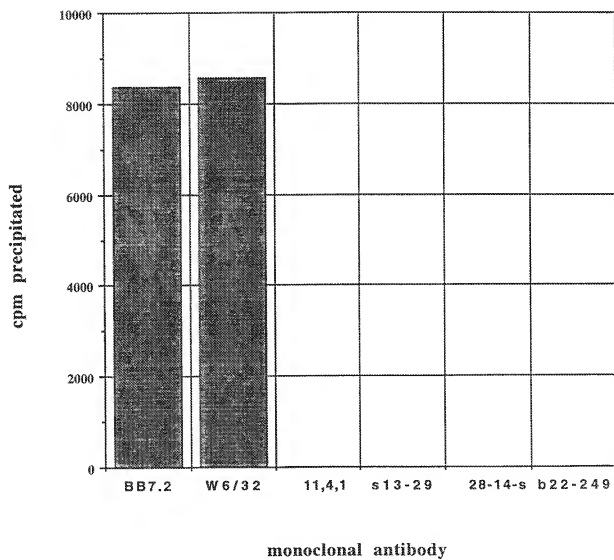
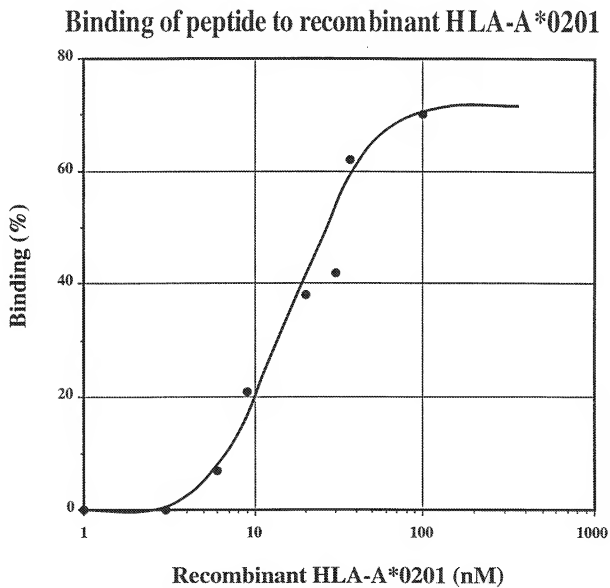


Fig. 5



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**Fig. 6**

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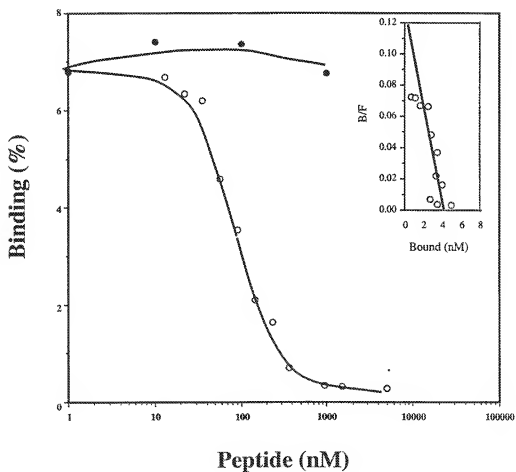
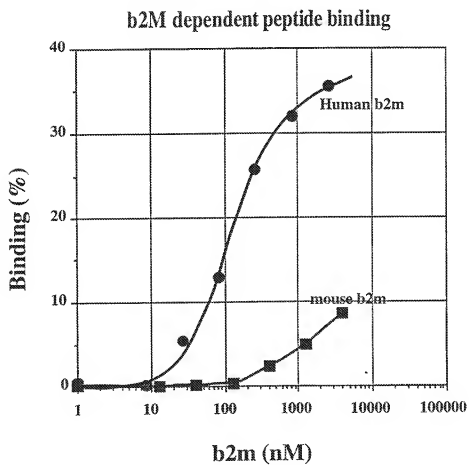


Fig. 7

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**Fig. 8**

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Binding of radiolabelled b2m to purified rA2.

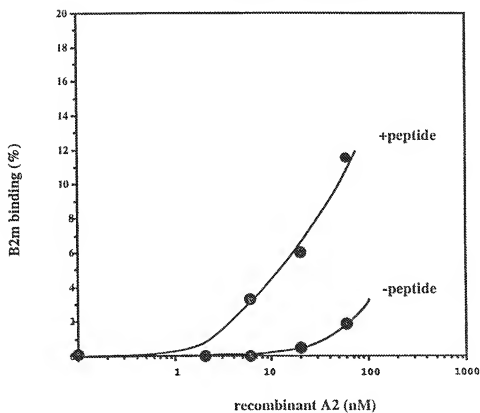
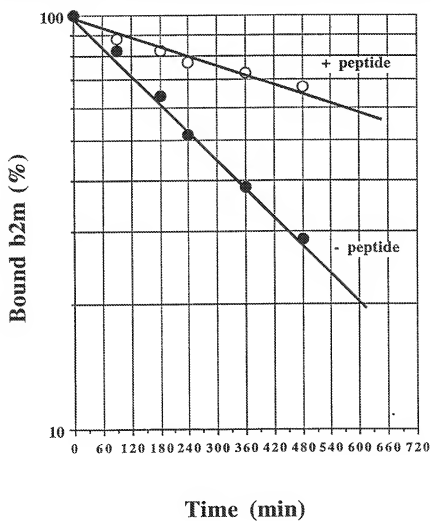


Fig. 9

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**Dissociation of labeled b2m from MHC class I.****Fig. 10**

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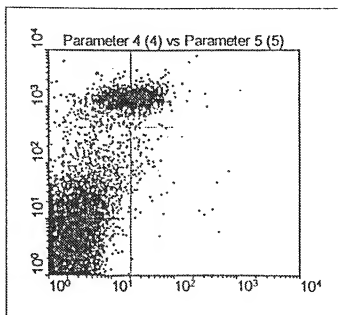


Fig. 11A

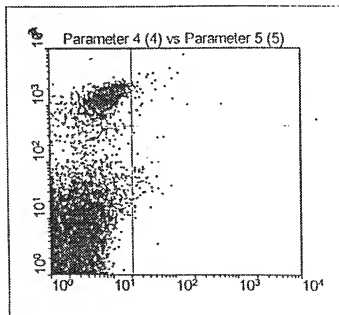
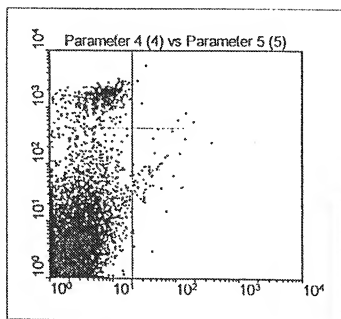
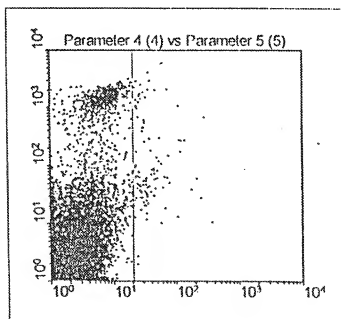


Fig. 11B

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**Fig. 11C****Fig. 11D**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup>:</b> <b>C07K 14/705, C12P 21/02, G01N 33/68</b> <b>// (C12P 21/02, C12R 1:19)</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 00/15665</b>  <b>(43) International Publication Date:</b> 23 March 2000 (23.03.00)
<b>(21) International Application Number:</b> PCT/DK99/00484  <b>(22) International Filing Date:</b> 14 September 1999 (14.09.99)  <b>(30) Priority Data:</b> PA 1998 01155 14 September 1998 (14.09.98) DK  <b>(71)(72) Applicants and Inventors:</b> PEDERSEN, Lars, Østergaard [DK/DK]; Ved Viglen 14, DK-2400 Copenhagen NV (DK). BUUS, Søren [DK/DK]; Stenmaglevej 29, DK-2700 Brønshøj (DK).  <b>(74) Agent:</b> PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 17 August 2000 (17.08.00)

(54) Title: A METHOD OF PRODUCING A FUNCTIONAL IMMUNOGLOBULIN SUPERFAMILY PROTEIN

## (57) Abstract

The present invention relates to a process of producing a functional immunoglobulin superfamily protein, which has at least one disulphide bond when functional, the process comprising the steps of providing a bacterial cell, where the gene is expressible, isolating the protein from the cell without reducing it and subjecting the isolated protein to a folding treatment. Preferably, the immunoglobulin superfamily protein is selected from the group consisting of Class I and Class II histocompatibility molecules and beta 2 microglobulin (beta2m). Other embodiments of the invention is a stable peptide free MHC protein obtainable by a process of the invention and a kit comprising a MHC class I heavy chain and a abeta2m allowing the recipient to produce and measure or detect a functional MHC class I protein to which a peptide, which is capable of binding to said MHC class I protein, can be added leading to the generation of a functional MHC class I protein.



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Int. J. Appl. No.

PCT/DK 99/00484

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/705 C12P21/02 G01N33/68 //(C12P21/02, C12R1:19)

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A	---	1-41,49
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A	PARKER K C ET AL: "An HLA-A2/beta 2-microglobulin/peptide complex assembled from subunits expressed separately in Escherichia coli." MOLECULAR IMMUNOLOGY, (1992 MAR) 29 (3) 371-8., XP000876800 cited in the application abstract page 372, right-hand column, paragraph 2 -page 373, left-hand column, paragraph 1  ---	1-42, 49
A	PLAKSIN D ET AL: "A T cell receptor V alpha domain expressed in bacteria: does it dimerize in solution?" JOURNAL OF EXPERIMENTAL MEDICINE, (1996 OCT 1) 184 (4) 1251-8., XP000876791 abstract page 1252, right-hand column, paragraph 2 -page 1253, left-hand column, paragraph 1  ---	1-42, 49
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A	<p>WO 93 22332 A (UNIV TEXAS ;WARD ELIZABETH  SALLY (US); KIM JIN KYOO (US))  11 November 1993 (1993-11-11)  page 6, line 25 -page 7, line 19  page 43, line 1-4</p>	1-42,49
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